Pestic. Sci. 1997, **50**, 83–84

Extended Summary
New Perspectives in Mechanisms of Herbicide Action

The following is an extended summary based on a paper presented at the symposium 'New Perspectives in Mechanisms of Herbicide Action' organised by D. J. Cole and A. H. Cobb on behalf of the Pesticides Group and held at 14/15 Belgrave Square, London, on 13 March 1996. It is entirely the responsibility of the authors and does not necessarily reflect the views of the Editorial Board of Pesticide Science.

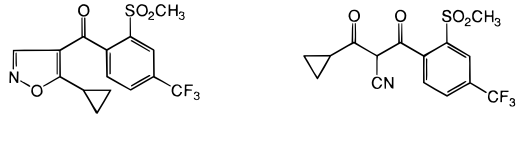
Inhibition of 4-Hydroxyphenylpyruvate Dioxygenase: the Mode of Action of the Herbicide RPA 201772 (Isoxaflutole)

K. E. Pallett, J. P. Little, P. Veerasekaran & F. Viviani

Rhone-Poulenc Agriculture Ltd, Fyfield Road, Ongar, Essex, CM5 0HW, UK

The benzoyl isoxazole herbicide RPA 201772, common name isoxaflutole (Fig. 1), is a novel product being developed for pre- and early post-emergence weed control in maize and sugarcane.[1] In plants and soil the isoxazole ring opens, forming a diketonitrile derivative (Fig. 1).[2] This is likely to be the active herbicidal principle of isoxaflutole, as it is a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD) in plants. Furthermore, the subsequent metabolic degradation of the diketonitrile occurs more rapidly in tolerant species such as maize and this appears to be the basis for herbicidal selectivity.[2]

Isoxaflutole causes a bleaching symptomology in susceptible species similar to that seen with herbicidal inhibitors of carotenoid biosynthesis, e.g. deflufenican and other phytoene desaturase (PDS) inhibitors. Coincident with decreases in carotenoid levels following isoxaflutole treatment is an accumulation of the PDS substrate, phytoene. Isoxaflutole and its diketonitrile derivative were tested for their ability to inhibit PDS isolated from cultured carrot cell microsomes. At concentrations up to 100 μM neither compound significantly inhibited PDS activity, whereas standards such as diflufenican and flurtamone had IC_{50} values of 100 and 400 nM respectively. Therefore, the accumulation of phytoene in treated leaves and bleaching symptoms appears to be due to an indirect effect on PDS.



ISOXAFLUTOLE DIKETONITRILE OF ISOXAFLUTOLE

Fig. 1. The structures of isoxaflutole and its diketonitrile derivative.

HPPD catalyses the oxidative decarboxylation of 4-hydroxyphenylpyruvate forming homogentisate. The reaction mechanism, which is still not fully understood, involves ring peroxidation, leading to ring hydroxylation and side chain migration.[3,4] Homogentisate then undergoes prenylation and methylation forming isoprenoid quinones required in biological redox reactions, such as plastoquinone. In bleached leaves levels of plastoquinone are depleted in advance of carotenoids. For example, HPLC analysis of *Brassica kaber* Wheeler seedlings revealed 40 and 75% decrease in plastoquinone 24 and 48 h after treatment with 63 g ha^{-1} isoxaflutole. Carotenoid levels were identical to untreated controls after 24 h and were decreased by 35% after 48 h when bleaching became visible. Furthermore, accumulation of phytoene became apparent after 48 h. It is suggested that inhibition of HPPD results in an indirect effect on carotenoid biosynthesis due to the depletion of plastoquinone, a proposed cofactor of PDS.

HPPD is a low-abundance enzyme in plants but it has now been purified and characterized from cultured carrot cells.[5] An assay has been developed involving the

Pestic. Sci. 0031-613X/97/$17.50

HPLC determination of homogentisate from 4-hydroxyphenylpyruvate in the presence of ascorbate.[6] The diketonitrile derivative of isoxaflutole is a potent inhibitor of this HPPD preparation with an IC_{50} of 5 nM. Initial studies into the kinetics of HPPD inhibition reveal that it is a slow tight-binding inhibitor of purified enzyme.[6]

REFERENCES

1. Luscombe, B. M., Pallett, K. E., Loubiere, P., Millet, J. C., Melgarejo, J. & Vrabel, T. E., RPA 201772 a novel herbicide for broadleaf and grass weed control in maize and sugarcane. *Proc. Brighton Crop Protection Conf.—Weeds*, **1** (1995) 35–42.
2. Little, J. P., Veerasekaran, P., Viviani, F. & Pallett, K. E., The mode of action and mechanism of selectivity of isoxaflutole (in preparation).
3. Lindblad, B., Lindstedt, G. & Lindstedt, S., The mechanism of enzymic formation of homogentisate from *p*-hydroxyphenylpyruvate. *J. Am. Chem. Soc.*, **92** (1970) 7446–9.
4. Pascal, R. A., Olivier, M. A. & Jack Chen, Y. C., Alternate substrates and inhibitors of bacterial 4-hydroxyphenylpyruvate dioxygenase. *Biochemistry*, **24** (1985) 3158–65.
5. Lenne, C., Matringe, M., Rolland, A., Sailland, A., Pallett, K. E. & Douce, R., Localisation and characterisation of 4-hydroxyphenylpyruvate dioxygenase from cultered carrot cells (in preparation).
6. Viviani, F., Little, J. P., Rodgers, M. W. & Pallett, K. E., Herbicidal inhibition of *p*-hydroxyphenylpyruvate dioxygenase by isoxaflutole (in preparation).